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PROTEIN KINASE C-ZETA IS INVOLVED IN IL-1/TNF SIGNALING PATHWAYS IN CHONDROCYTES

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Aim of Study: In osteoarthritis (OA), catabolic factors like Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) have a direct effect on chondrocytes by stimulating the production of matrix degrading enzymes including Aggrecanases and metalloproteases resulting in proteoglycan and collagen degradation, respectively. Protein Kinase C (PKC) is a family of serine/threonine kinases divided into three major groups: conventional, novel and atypical. PKC-zeta (PKCz) is a member of the atypical PKC and controls important cellular functions through the regulation of critical signaling pathways. The aim of this study is to understand the role of PKCz in IL-1/TNF pathways in chondrocytes.
Methods: *PKCz expression in cartilage:* RNA was extracted from frozen pulverized articular cartilage tissue from normal and OA specimens and subjected to gene expression profiling analysis. For further confirmation of gene expression, quantitative PCR analysis was performed.
NF-kB-luciferase cell line: Human chondrocyte cell line T/C-28a2 was transfected with NF-kB response elements coupled to luciferase gene and a stable cell line (T/C-NFkB-luci) was selected which responded to IL-1/TNF stimulation.
PKCz expression in chondrocytes: Primary chondrocytes were isolated from both bovine and human cartilage. Cells were stimulated with IL-1/TNF in the absence or presence of PKC inhibitors. Expression of aggrecanase was analyzed by quantitative PCR.
Results: *PKCz expression was increased in OA cartilage:* Results from gene expression profiling studies showed that in relation to normal cartilage, PKCz expression was increased in OA cartilage. The results were further confirmed using quantitative PCR.
PKCz inhibitors inhibited IL-1/TNF induced NF-kB: Human chondrocytic ell line, T/C-NFkB-luci stimulated with IL-1/TNF showed increased luciferase activity in the luciferase reporter assay indicating the activation of NF-kB in chondrocytes. Inhibitors of PKCz, but not PKCz-sparing inhibitors inhibited luciferase activity indicating the regulation of NF-kB signaling by PKCz.
Inhibition of PKCz downregulated aggrecanase expression: Primary chondrocytes stimulated with IL-1/TNF upregulated aggrecanase expression. Addition of PKCz inhibitors resulted in the downregulation of aggrecanase expression.
Conclusions: PKCz provides a unique opportunity to study the regulation of IL-1 and TNF signaling pathways in chondrocytes. Our results show that PKCz is involved in the regulation of IL-1/TNF induced NF-kB signaling which in turn regulates the downstream expression of aggrecanase. Therefore, inhibition of PKCz could potentially regulate the production of degradative enzymes and have a profound effect on the disease progression in OA.

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THE ISOFORMS OF ADAMTS-4 AND -5 VARY WITH IL-1 DOSE IN EXPLANT CULTURES

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Aims: ADAMTS-4 and -5 have been shown to be the enzymes responsible for aggrecan degradation in articular cartilage explant cultures treated with IL-1, and to exist as a number of isoforms with differing proteolytic activities and specificities. The objective of this study was to analyse ADAMTS-4 and -5 secretion and activation in articular cartilage explant cultures treated with varying doses of IL-1.
Methods: Porcine articular cartilage explants were cultured in DMEM with or without 10 or 100ng/ml IL-1 and harvested after 4, 7 and 10 days. The DMMB assay was used to measure GAG release and media samples were analysed for aggrecanase-generated IGD aggrecan catabolites by Western blotting with M'Ab BC-3. Isoforms of ADAMTS-4 and -5 in media samples were detected using M'Ab Anti-ADAMTS-4N, recognising the metalloproteinase domain of ADAMTS-4 and polyclonal antibody Anti-ADAMTS-5 Catalytic domain, respectively.
Results: GAG release to the medium was highest in cultures treated with 100ng/ml IL-1, and higher in cultures treated with 10ng/ml IL-1 than in Control cultures. Increased GAG release corresponded with increased detection of aggrecanase-generated IGD aggrecan catabolites by Western blotting using BC-3. ADAMTS-4 isoforms were detected at 45, 37 and 30kD using Anti-ADAMTS-4N. ADAMTS-5 isoforms were detected at 42, 37 and 30kD using Anti-ADAMTS-5 Catalytic domain. The 45kD isoform of ADAMTS-4 and the 42kD isoform of ADAMTS-5 were detected at equal intensity in all cultures. The co-migrating 37kD isoforms of ADAMTS-4 and -5 were detected at increased intensity in cultures treated with 10ng/ml IL-1. Interestingly, higher MW isoforms of ADAMTS-4 and -5 were detected (at 68 and 70kD, respectively) only in media samples from cultures treated with 100ng/ml IL-1. These higher molecular weight isoforms of ADAMTS-4 and -5 were detected at increased intensity with increasing time in the presence of 100ng/ml IL-1. However, these isoforms were not detectable in cultures treated with 10ng/ml IL-1 even after 10 days treatment.
Conclusion: The co-migrating 37kD isoforms of ADAMTS-4 and -5 detected at increased intensity in explants treated with 10ng/ml IL-1 suggests a role for these low MW isoforms in the IGD aggrecanase activity detected in these cultures. However, these co-migrating 37kD isoforms of ADAMTS-4 and -5 are not detected at higher levels in media samples from explants treated with 100ng/ml IL-1 than those treated with 10ng/ml IL-1, despite the detection of increased IGD aggrecanase activity in these cultures. This implies the higher MW, possibly full length furin-activated isoforms of ADAMTS-4 and -5 (68 and 70kD, respectively) detected in explants treated with 100ng/ml IL-1, to be in part responsible for the IGD aggrecanase activity detected in these cultures. This data indicates different doses of IL-1 may have differing effects on the isoforms of ADAMTS-4 and -5 present in explant cultures and their IGD aggrecanase activity